DAILY GROWTH INCREMENTS IN THE SAGITTA OF PILCHARD LARVAE *SARDINA PILCHARDUS* (WALBAUM, 1792), (PISCES : CLUPEIDAE)

by

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ABSTRACT. – Growth patterns attributed to daily increments were observed in the sagitta of *Sardina pilchardus* larvae sampled off Portugal. The enumeration of daily rings was used to determine the chronological age of a few field-caught larvae taking into account the timing of the initiation of daily growth increments.

RESUMÉ. – Nous avons pu observer dans les sagittae de larves de *Sardina pilchardus* capturées au large des côtes portugaises des lignes de croissance attribuées à des dépôts journaliers. Le compte des anneaux journaliers a été employé pour la détermination de l'âge chronologique d'un nombre réduit de larves en tenant compte du temps écoulé depuis le début de la formation des anneaux.

Introduction

Pannella (1971) was the first author to present indirect evidence of the presence of daily growth layers and periodical deposition patterns in the sagittae of a reduced number of fish species. In a later paper the same author (Pannella, 1974) confirmed the existence of daily growth layers in the sagittae of several species of tropical fishes, and suggested that these might constitute a mean for conducting age and growth studies.

Recent work on otoliths of larvae and juveniles of *Engraulis mordax* (Brothers *et al.*,, 1976) has shown that daily rings may be used to estimate its age with great precision up to 100 days. These workers reported also that the exact timing of the initiation of daily increments (before or after yolk absorption) must be independently determined for each species in laboratory reared specimens. Struhsaker & Uchiyama (1976) also proved the existence of daily growth increments in the otoliths of another engraulid fish from the Hawaiian islands area.

Otoliths grew by addition of layers of material differing in the relative amount of the protein (otolin) and calcium carbonate in the aragonite form (Degens *et al.*, 1969 ; Pannella, 1971). This results in growth units or increments, that can be deposited on a daily or sub-daily basis, composed of an inner light band and an outer dark band. Since a similar pattern of deposition was observed in the sagittae of pilchard larvae, growth increments will be refered here-after as daily units.

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The causative factors of the daily lines that can be observed in the sagittae of **fishes** are, at the present state of knowledge, difficult to point out. Pannella (1974) refers that rhytmic growth is controlled by environmental changes geared to the diurnal astronomical cycle, temperature, food and light being amongst the factors that have a direct influence on the formation and development of daily growth units.

The aim of the present paper is to demonstrate the presence of growth rings attributed to daily increments in the otoliths of pilchard larva and to point out their use in age determination of field-caught specimens.

Material and Methods

Larvae of *Sardina pilchardus* were sampled over a grid of 10 stations in the inshore waters of the central part of the Portuguese coast, using a 1 meter ring trawl of 0.500 mm mesh aperture, hauled on an oblique tow during 10 minutes, at a constant speed of 2 knots. Sampling was carried only during daylight hours (between 10:00 and 15:00 local time) in January 1982. Fixation and preservation were made with 5 % formalin buffered with borax (pH 8.5).

Sagittae were obtained from the larva, after a period of two month preservation in formalin, by placing the specimen on a slide and extracting them from the head region with the aid of fine forceps and needles. These were then mounted permanently in a quick-drying neutral mounting medium (DEPEX). It was not necessary to ground and polish the sagittae as the growth increments were directly evident with under lightning on the microscope. The otoliths having a planoconvex form were mounted with their plane face (= internal face) upwards, because it was found that the enumeration of daily rings was easily made this way. Growth rings were counted with the aid of a microscope using magnifications ranged from 400 to 1000 x. Each sagitta was counted several times and verification counts were also made at a later time. Sagitta diameters were measured with a calibrated eyepiece to the nearest µm. Total length of larvae was taken to the nearest 0.5 mm. We did not took into account larval shrinkage due to fixation and preservation in formalin.

Results

Daily growth increments were enumerated from the sagittae of 42 larvae. The length-frequency distribution of these larvae is shown in figure 1.

Daily rings were perfectly seen in the sagittae of larvae which were fixed and preserved for about two months in formalin buffered with borax to pH 8.5. Growth increments were also clearly enumerated in the sagittae of postlarvae after a period of preservation of two years in buffered formalin. This apparently contradicts the observation of other authors. Struhsacker & Uchiyama (1976) refer that the otoliths of a fresh specimen tended to clear completely within a few hours.

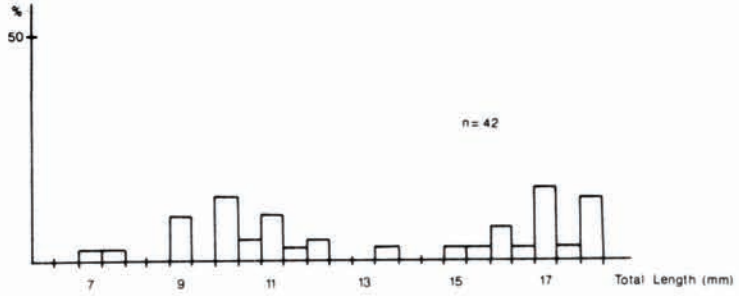

Fig. 1. – Length – frequency distribution of the larvae examined.

(after being mounted in a clear mounting medium) so that photographs were the only permanent record of daily increments.

The determination of the chronological age of a larval specimen has to take into account the exact timing of the initiation of daily growth increments. Brothers *et al.* (1076) found that daily increments appeared in the otolith of *Engraulis mordax* only after the completion of yolk-sac absorption. The sagittae of these larvae have a clear nucleus (without any apparent rings) and the growth increments were first deposited after a period of 5 days.

The fact that the sagittae of *S. pilchardus* larvae had the same appearance led us to believe that we were in the presence of a similar situation. The confirmation of this hypothesis implies experimental rearing work. As we did not have the facilities to conduct rearing experiments we adopted a period of 4 days for the yolk sac period (Blaxter, 1969 gives a period of 3 to 5 days between 17 and 10°C). The chronological age of a given larva thus can be determined by adding 4 days to the number of rings enumerated in the sagitta.

Daily rings were counted in the sagittae of pilchard larvae using magnifications of 1000 x in oil immersion. The two sagittae were normally used in age determina-

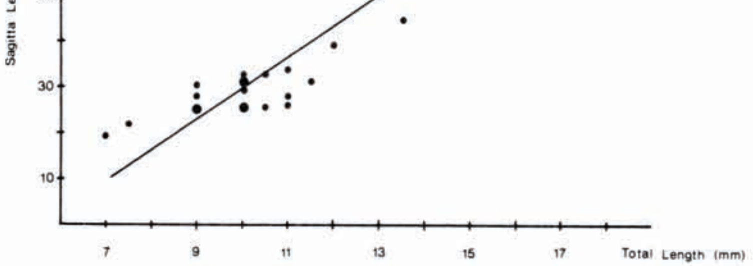

Fig. 2. – Correlation between total length of the larvae and length of the sagittae.

tion for each larva. Daily ring thickness varied from 0.5 to 1 μm. The smallest growth increments present near the nucleus were the most difficult to enumerate.

Very good correlations ($p = 0.001$) were found between total length of the larva and sagitta length ($r = 0.974$, $n = 37$) (figure 2), sagitta length and daily growth increments ($r = 0.971$, $n = 34$) (figure 3), total length of the larva and daily growth increments ($r = 0.973$, $n = 37$) (figure 4).

The chronological age of the larvae observed is given in Table I.

Conclusions

The daily increments present in the sagitta of larval fishes may be used to determine with great precision its chronological age even after a prolonged period of preservation in buffered formalin. To achieve this we have to take into account the exact timing of the initiation of daily growth increments. In the case of *Sardina pilchardus* daily rings seem to appear only after the completion of yolk-sac absorption, and very good correlations were found between these increments and other characters as the sagitta length and the total length of the larva.

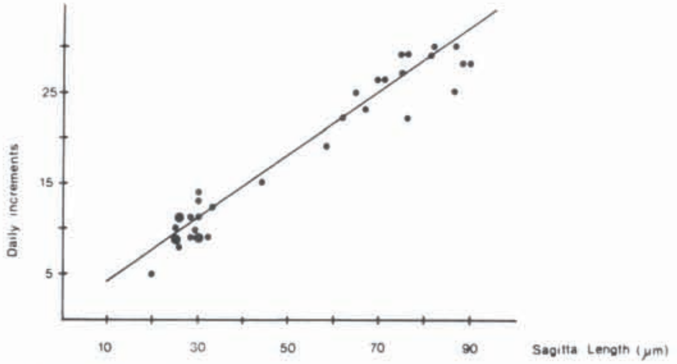

Fig. 3. – Correlation between length of sagittae and number of increments.

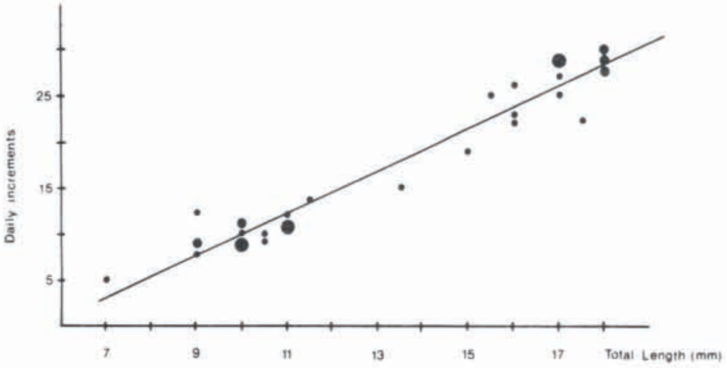

Fig. 4. – Correlation between total length of the larvae and number of increments.

Acknowledgements

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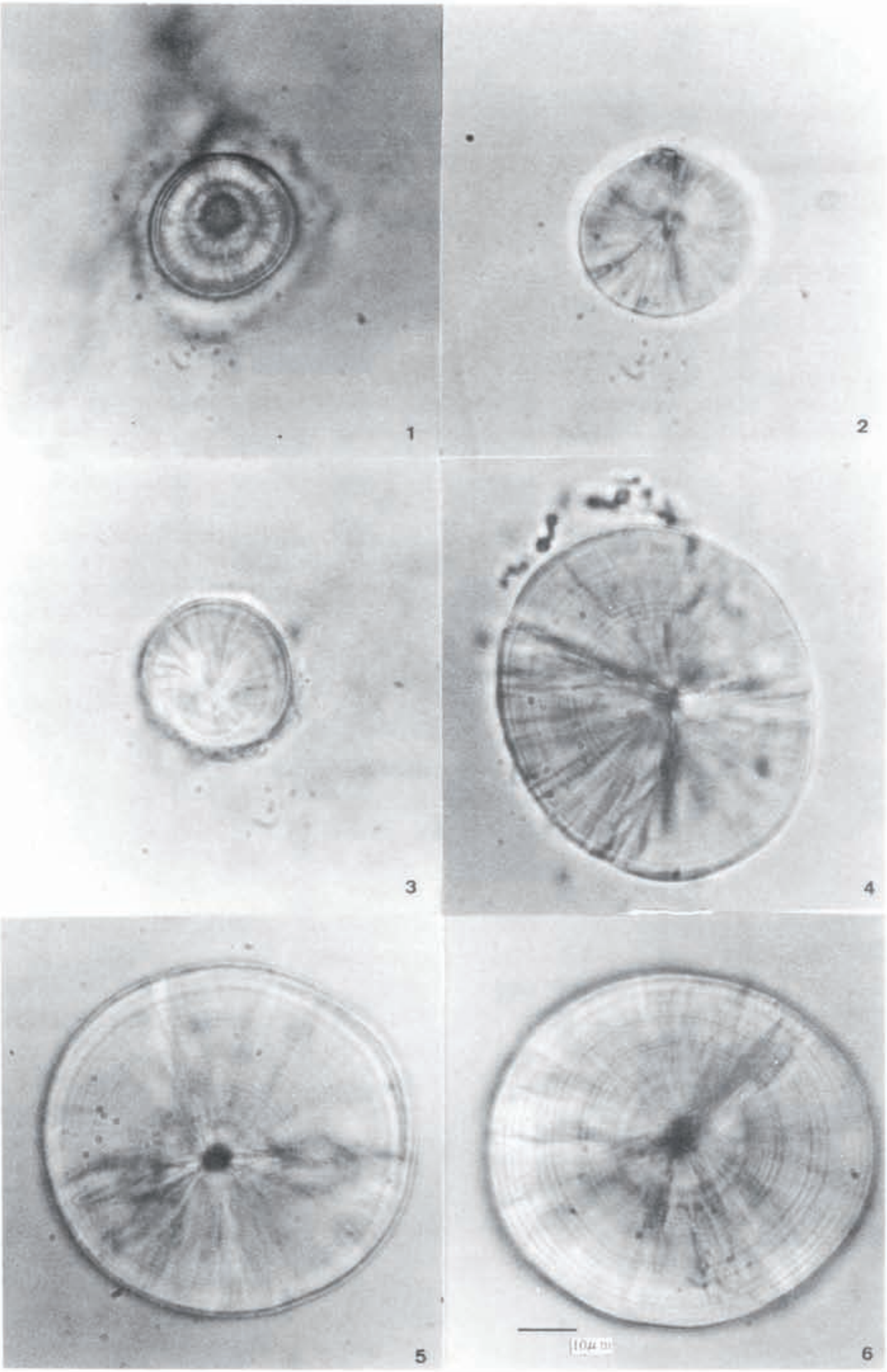
Table I. – Chronological age (from hatching) and number of daily growth increments of *S. pilchardus*.

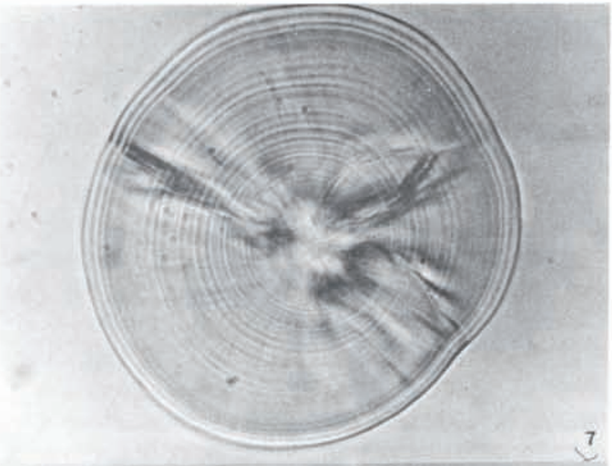
Total length (mm)	Number of larvae	Mean number of increments	Range	Chronological age (days)
7	1	5	5	9
9	4	10	9-13	14
10	6	10	9-11	14
10.5	2	10	9-10	14
11	4	11	11-12	15
13.5	1	15	15	19
15	1	19	19	23
15.5	1	25	25	29
16	3	24	22-26	28
17	6	28	25-29	32
18	6	29	28-30	33



Photomicrographs of sagittae of *Sardina pilchardus* larvae

(TL: total length of the larva ; sL: sagitta length ; R: number of daily increments ; A: chronological age).

1 – TL : 10.0 mm ; sL : 25.2 μm ; R : 10 ; A : 14 days.
2 – TL : 10.0 mm ; sL : 30.8 μm ; R : 10 ; A : 14 days.
3 – TL : 11.0 mm ; sL : 28.0 μm ; R : 11 ; A : 15 days.



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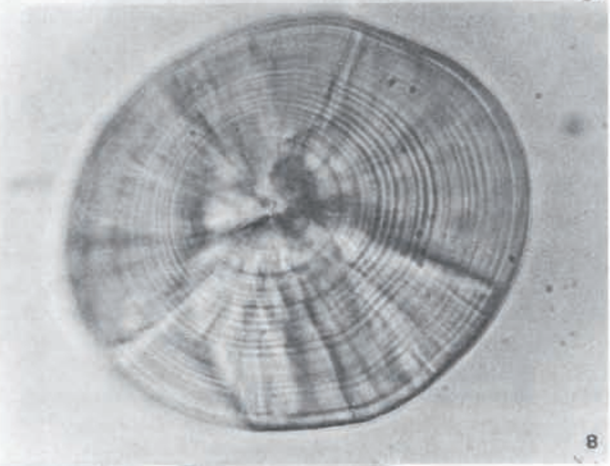

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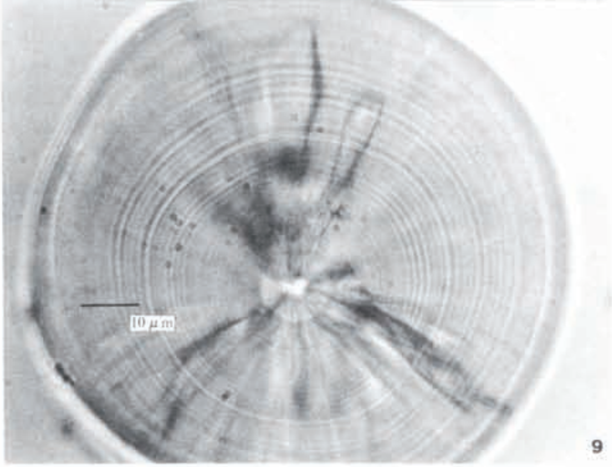

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4 – TL : 15.0 mm ; sL : 58.8 µm ; R : 19 ; A : 23 days.
5 – TL : 15.5 mm ; sL : 64.6 µm ; R : 25 ; A : 29 days.
6 – TL : 16.0 mm ; sL : 67.2 µm ; R : 23 ; A : 27 days.
7 – TL : 17.0 mm ; sL : 75.6 µm ; R : 29 ; A : 33 days.
8 – TL : 18.0 mm ; sL : 81.2 µm ; R : 29 ; A : 33 days.
9 – TL : 18.0 mm ; sL : 89.6 µm ; R : 29 ; A : 33 days.